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291

NOVEL APPROACH FOR PREDICTING GVHD REACTIVE T CELLS USING VB SPECTRATYPE ANALYSIS OF DIVIDING CFSE-LABELED T CELLS FROM A MIXED LYMPHOCYTE REACTION

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The major complication associated with allogeneic hematopoietic stem cell transplantation (HSCT) is graft-versus-host disease (GVHD), which is mediated by mature T cells in the donor transplant inoculum. Since donor T cells are also important in preventing graft failure, opportunistic infections and leukemia relapses after transplant, it would be highly advantageous to identify and separate T cells with GVHD-reactivity from those capable of mediating graft-versus-leukemia (GVL) responses. In this regard, TCR Vβ repertoire analysis by CDR3-size spectratyping can be a powerful tool to characterize alloreactive T cell responses. As an initial test of this capability, we studied lethal GVHD in the B6 -> CXB-2 MHC-matched, minor histocompatibility antigen disparate murine model to determine the correlation between the in vitro detected alloreactive B6 $CD8^+$ T cell responses and their actual involvement in the development of GVHD upon transfer to lethally irradiated CXB-2 recipients. Previous methods to obtain the Vβ repertoire from responding mixed lymphocyte reactions (MLR) required expansion of the alloreactive cells in secondary stimulation cultures, for a total of 20 days. To significantly shorten this incubation period, in order to eventually adapt this technique for clinical use, we used the fluorescent-based intracellular dye CFSE to label responding cells in a 4-day MLR and fluorescent cell sorting to isolate the divided alloreactive cells that could then be processed for Vβ spectratype analysis. In a comparative study of the predictive value of the Vβ repertoire detected in traditional MLR versus CFSE/MLR, the results indicated that the former approach correlated with 78% of the Vβ CDR3-size skewing patterns observed in mice with B6 -> CXB-2 GVHD, and the CFSE/MLR approach corresponded to 65% of those skewed Vβ families found in vivo. These results suggest that in vitro spectratyping analysis, and particularly the use of CFSE to predict in vivo reactivity, may be a useful technique in eventually guiding the manipulation of the donor T cell inoculum in order to improve the outcome of HSCT.

292

DENDRITIC CELL VACCINATION EXPANDS ANTIGEN SPECIFIC T CELLS FOLLOWING ALLOGENEIC TRANSPLANTATION

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Background: The graft versus malignancy (GVM) effect after allogeneic hematopoietic stem cell transplantation (alloHSCT) is the most potent form of cancer immunotherapy. However, disease recurrence remains a major contributor to poor outcome and non-specific strategies to enhance GVM responses are frequently complicated by GVHD. Dendritic cell (DC) vaccines may be a strategy to increase the potency and specificity GVM responses but the impact of alloreactivity on DC vaccine responses has not been established. **Methods:** We tested DC vaccines in a parent (C57BL/6) into F1 (C57BL/6xC3H.SW) MHC-matched T cell-depleted (TCD) transplant. Female recipients received donor lymphocytes (DLI) with a DC vaccine targeting the male antigenic complex (HY) on days 14 and 28. Responses were measured using ELISPOT and MHC class I tetramer on day 42. **Results:** Mice receiving TCD bone marrow with low dose of DLI (1×10^6) show no GVHD-associated weight loss. Higher dose DLI ($5\text{-}20\times10^6$) induces weight loss without lethality. DC vaccination of allogeneic recipients plus low dose DLI generates quantitatively similar dominant CD8 and CD4 vaccine responses to syngeneic controls with increased subdominant CD8 responses (15×10^4 vs 7×10^4 cells/spleen, $p=0.06$). GVHD induction with higher doses of DLI results in loss of vaccine responses. We next explored whether responses can be generated in the absence of thymic T cell regeneration since this pathway may be limited following alloHSCT. Equivalent HY responses to thymus-bearing recipients are generated in syngeneic, thymectomized recipients but requires high dose (20×10^6) DLI as a source for peripheral expansion. Allogeneic recipients receiving high dose DLI are unable to respond to HY vaccination due to GVHD. 10×10^6 8-methoxypsoralen and ultraviolet B light-induced apoptotic donor- or recipient-type spleenocytes infused at the time of DLI (to model extracorporeal photopheresis (ECP)) prevents GVHD even in mice receiving high dose DLI. Importantly, modulation of GVHD by ECP preserves vaccine-mediated HY responses even in recipients lacking a thymus. **Conclusions:** DC vaccines can expand antigen-specific T cells following alloHSCT. GVHD adversely affects these responses and precludes the induction of DC vaccine responses in the absence of thymic function. Modulation of GVHD by ECP preserves vaccine responses to non-alloantigens. This approach may provide an opportunity to selectively enhance graft versus tumor responses following alloHSCT.

293

RISK FACTORS ASSOCIATED WITH CHRONIC GVHD REVISITED: A STUDY OF 142 PATIENTS WITH MALIGNANT DISEASES TRANSPLANTED IN A SINGLE CENTER IN BRAZIL

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Chronic graft-versus-host disease is a well known cause of transplant related mortality after HSCT. OBJECTIVE: evaluate the impact chronic GVHD at the overall survival and disease free survival among 142 patients who received transplants for malignant diseases and developed chronic graft-versus-host-disease from 07/95 to 06/05 at the BMT center of HC-UFPR in Curitiba, Brazil. PATIENTS CHARACTHERISTICS: male: 94; female: 48; median age 31 years (range: 1-54). Cell source was bone marrow in 134 patients, cord blood in four patients and pheripheral blood in four patients. Conditioning regimen was nomyeloablative in 3 patients and conventional in 139 patients. Immunoprophylaxis: CSA + MTX + CTC = 76; CSA+MTX =54; CSA without MTX +/- CTC= 8 patients. HLA compatible: 120 patients, mismatch class I : 10, class II : 6, more than one mismatch : 2. Thirty three patients received HSCT from unrelated donors, 11 from related alternative donors and 98 from siblings. Female donor / male recipient in 47 patients. Median duration of disease: 31 months. Diagnosis: CML 79, AML/MDS 44; ALL 15, OTHERS 4.

RESULTS: Overall survival was 67% in 10 years. Median survival 2181 dias (82-4575). Risk factors with significance for survival were Platelets less than 100,000 /mm3 at diagnosis, and lymphocytes less than 1,500/mm3. There was a tendency for TB more than 1.2 at diagnosis. There was no significance for age, gender, presence of female donor/male patient or HLA compatibility.

CONCLUSIONS: This study confirmed previous data and identified thrombocytopenia and lymphopenia at the diagnosis as risk factors for survival in patients with chronic graft-versus-host disease.

294

ALEMTUZUMAB IN THE TREATMENT OF STEROID REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE

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Corticosteroids therapy is the mainstay of treatment for GVHD, however, it heavily impacts on post transplant morbidity and new modalities are continually needed. Alemtuzumab a humanized monoclonal antibody to CD52 has been used mainly as GVHD prophylaxis. Only a few patients have been treated with this antibody. From December 2004 to May 2006, we recruited 13 steroid refractory grade II-IV acute GvHD patients in a prospective trial evaluating the efficacy of alemtuzumab (Campath 1H) after exclu-